AWARD NUMBER: **W81XWH-12-2-0025**

TITLE**: Effect of Antimicrobial Peptide KSL-W on Human Gingival Tissue and *C. albicans* Growth, Transition and Secreted Aspartyl Proteinase (SAPS) 2, 4, 5 and 6 Expressions.**

PRINCIPAL INVESTIGATOR: **Dr Mahmoud Rouabhia, Dental Faculty, University of Laval**

CONTRACTING ORGANIZATION: **University of Laval**
QUEBEC, CA G1V 0A6

REPORT DATE: April 2014

TYPE OF REPORT: **Annual Report**

PREPARED FOR: **U.S. Army Medical Research and Materiel Command**

Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
April 2014	Annual	1 Apr 2013 - 31 Mar 2014

4. TITLE AND SUBTITLE
Effect of Antimicrobial Peptide KSL-W on Human Gingival Tissue and C. albicans Growth, Transition and Secreted Aspartyl Proteinase (SAPS) 2, 4, 5 and 6 Expressions.

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-2-0025

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr Mahmoud Rouabhia

Email: mahmoud.rouabhia@fmd.ulaval.ca

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Laval
QUEBEC, CA G1V 0A6

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The antifungal armamentarium for the treatment of systemic fungal infections has increased in recent years. Although very helpful to control/eliminate fungal infections, the available antifungal drugs do have some limitations such as antifungal drug resistance. As an example, azole resistance is an issue in patients with chronic mucocutaneous candidiasis caused by C. albicans in the context of HIV-infected individuals with recurrent oropharyngeal and esophageal candidiasis. A similar trend in vaginal isolates of C. albicans has been seen in women prone to recurrent vaginal candidiasis exposed to long-term fluconazole (Bulik et al., 2009),(Shahid and Sobel, 2009). In the latter scenario – fortunately relatively rare to date – therapeutic options available for oral management of fluconazole-reduced susceptibility C. albicans are few, resulting in the inconvenient use of long-term topical imidazoles. These facts have generated greater interest in the development of new antifungal drugs using various synthetic and naturally occurring antimicrobial molecules. Natural antimicrobial peptides, such as defensins produced by epithelial cells, showed a broad range of antibacterial activity and could play a role in preventing microbial infections(Decanis et al., 2009),(Zaslof, 2002). These antimicrobial peptides generally exhibit selective toxicity for microorganisms and show fewer propensities to induce microbial resistance.

15. SUBJECT TERMS- nothing listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU		19b. TELEPHONE NUMBER *(include area code)*

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1-INTRODUCTION: The antifungal armamentarium for the treatment of systemic fungal infections has increased in recent years. Although very helpful to control/eliminate fungal infections, the available antifungal drugs do have some limitations such as antifungal drug resistance. As an example, azole resistance is an issue in patients with chronic mucocutaneous candidiasis caused by *C. albicans* in the context of HIV-infected individuals with recurrent oropharyngeal and esophageal candidiasis. A similar trend in vaginal isolates of *C. albicans* has been seen in women prone to recurrent vaginal candidiasis exposed to long-term fluconazole (Bulik *et al.*, 2009)(Shahid and Sobel, 2009). In the latter scenario – fortunately relatively rare to date – therapeutic options available for oral management of fluconazole-reduced susceptibility *C. albicans* are few, resulting in the inconvenient use of long-term topical imidazoles. These facts have generated greater interest in the development of new antifungal drugs using various synthetic and naturally occurring antimicrobial molecules. Natural antimicrobial peptides, such as defensins produced by epithelial cells, showed a broad range of antibacterial activity and could play a role in preventing microbial infections(Decanis *et al.*, 2009)(Zaslof, 2002). These antimicrobial peptides generally exhibit selective toxicity for microorganisms and show fewer propensities to induce microbial resistance.

***Scope of the research* :** For the development of alternative antifungal treatment, we have synthesized an α-helical antimicrobial decapeptide, KSL (KKVVFKVKFK), and its analog, KSL-W (KKVVFWVKFK)(Na *et al.*, 2007), which possess a broad range of antibacterial activity. It killed selected strains of non-oral and oral pathogens, including mutans streptococci. In combination with a surface-active agent, benzalkonium chloride, the peptide significantly reduces *in vitro* biofilm growth(Dixon *et al.*, 2008; Dixon *et al.*, 2009; Leung *et al.*, 2005; Leung *et al.*, 2009).

2-KEYWORDS: Fungal treatment, *C. albicans*, Antifungal molecules, fungi resistance, antimicrobial peptides, cationic peptides, chemical peptides, KSL-W.

3-**ACCOMPLISHMENTS:** There was no change as to the original proposal.

The primary goals of this study were:

1. To investigate the effect of antimicrobial peptide KSL-W (developed by the US Army Dental and Trauma Research Detaachment) on *C. albicans* growth and biofilm formation under the activation of virulence genes.
2. To investigate the effect of KSL-W on human gingival cell growth and migration/wound healing.

Accomplished work

1) **Major activities:** We conducted a complete study evaluation the effect of KSL-W on *C. albicans* growth and pathogenesis.

2) **We specifically** studied the *C. albicans growth, transition and virulence gene (*EFG1, NRG1, EAP1, HWP1, and SAP 2-4-5-6) expression following yeast contact with KSL-W.

3) ***Results:*** We demonstrated that KSL-W markedly reduced *C. albicans* growth at both early and late incubation times. The significant effect of KSL-W on *C. albicans* growth was observed beginning at ten μg/ml after five h of contact by reducing *C. albicans* transition and at 25 μg/ml by completely inhibiting *C. albicans* transition. Cultured *C. albicans*under biofilm-inducing conditions revealed that both KSL-W and amphotericin B significantly decreased biofilm formation at 2, 4, and six days of culture. KSL-W also disrupted mature *C. albicans* biofilms. The effect of KSL-W on *C. albicans* growth, transition, and biofilm formation/disruption may thus occur through gene modulation, as the expression of various genes involved in *C. albicans* growth, transition and biofilm formation were all down-regulated when *C. albicans* was treated with KSL-W. The effect was greater when *C. albicans* was cultured under hyphae-inducing conditions. These data provide new insight into the efficacy of KSL-W against *C. albicans* and its potential use as an antifungal therapy.

4) ***Other achievements.*** We did not perform the studies related to the second objective because the lack of funding. There was no funding transferred from the funding agency to the university to do the study.

All needed information's related to the different protocols we used, and the figures about to the results are included in the published paper (see appendix 1). As a conclusion, we clearly demonstrated the efficacy of KSL-W on influencing *C. albicans* growth, phase transition and expression of virulence genes. This suggested the usefulness of KSL-W against *C. albicans* pathogenesis. However, the use of KSL-W for clinical applications should first be supported by *in vitro* studies using human cells to confirm the non-toxicity of the peptide.

Opportunities for training:

1. A student was involved in the project under his Master degree achievement. He was involved in the experimental protocols with *C. albicans*, data collections, and manuscript preparation.
2. The student contributed in presenting the work on antimicrobial peptide KSL-W on the research day of the Faculty of Dentistry, and at the Medical faculty of Laval University.

Results dissemination:

The results were disseminated through publications and presentations.

Plan for the next reporting period:

If the money transfer occurs to perform the second objective, we will be more than happy to perform the study related to objective 2 investigating the effect of KSL-W on human gingival cell growth and migration/wound healing.

4-IMPACT: The major accomplishment is the understanding the mechanism(s) by which antimicrobial peptide KSL-W in reducing *C. albicans* pathogenesis *in vitro*.

The impact on the development of the principal discipline(s) of the project

We clearly demonstrated that KSL-W was effective in reducing *C. albicans* growth, transition through the down-regulation of certain important genes involved in biofilm formation. This

consolidates the previous studies on inhibition of bacterial growth and suggests the potential use of KSL-W against microbial infections in human.

The impact on other disciplines
Nothing to Report.

The impact on technology transfer
Nothing to Report.

The impact on society beyond science and technology
Eventually the data generated through the first objective may suggest the use of KSL-W to control infection and minimize the emergence of microbial resistance. Such improvement may be of great economic improvement in reducing infection and promoting person health. This will allow more active work, thus economic improvement. It may also be very important for the design of new antimicrobial molecules, thus giving good treatment alternative, and creating more jobs.

5-CHANGES/PROBLEMS:
Nothing to report.

Changes in approach and reasons for change
Nothing to report.

Changes that had a significant impact on expenditures
There was no delay in performing the first objective. Thanks to the University Laval Financial department for providing support in advance to pay for different expenses and the studentship. This allowed us to perform most of the experiments on time which led to collection of useful data and the publication of a peer-reviewed paper. The second objective could not be performed, because there was lack of funds coming from the funding agency to the University Laval.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to Report.

Significant changes in use or care of human subjects
Nothing to Report.

Significant changes in use or care of vertebrate animals.
Nothing to Report.

Significant changes in use of biohazards and/or select agents.
Nothing to Report.

6-PRODUCTS:
Nothing to Report.

Publications, conference papers, and presentations

Journal publications.

Theberge S, Semlali A, Alamri A, Leung KP, **Rouabhia M**. C. albicans growth, transition, biofilm formation, and gene expression modulation by antimicrobial decapeptide KSL-W. BMC Microbiol. 2013 Nov 7;13:246. doi: 10.1186/1471-2180-13-246

Status of publication: Published

Acknowledgement of federal support: Yes

Abstracts:

1. Theberge Simon, Jacques Éric and Leung Kai P and Rouabhia Mahmoud. Un nouveau peptide antimicrobien contrôle la virulence de Candida en réduisant sa viabilité via un processus d'apoptose et de nécrose. Journée de la recherche GREB/FMD, le 10 mai, 2013

   Status of publication: Published in the event proceeding
   Presentation: Oral
   Acknowledgement of federal support: Yes

2. Théberge Simon, Jacques Éric, Leung Kai P and **Rouabhia Mahmoud**. Un nouveau peptide antimicrobien contrôle la virulence de Candida en réduisant sa viabilité via un processus d'apoptose et de nécrose. Journée de la recherche faculté de médecine – 30 mai 2013, Université Laval. Québec.

   Status of publication: Published in the event proceeding
   Presentation: Oral
   Acknowledgement of federal support: Yes

3. Théberge Simon, Semlali Abdelhabib, Alamri Abdullah, Leung P. Kai, and **Rouabhia Mahmoud**. Le KSL-W réduit la croissance de Candida albicans et la formation de biofilm en diminuant l'expression de plusieurs gènes de virulence. 81^{e} Congrès de l'Acfas, du 6 au 10 mai 2013, Université Laval, Québec, Canada.

   Status of publication: Published in the event proceeding
   Presentation: Oral
   Acknowledgement of federal support: Yes

Other publications, conference papers, and presentations.
None

Website(s) or other Internet site(s)
None

Technologies or techniques
None

Inventions, patent applications, and/or licenses
None

Other Products
None
7-PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

See below Tables.

Name:	Simon Theberge
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	University Laval Student
Nearest person month worked:	20 h a week
Contribution to Project:	M. Theberge has performed a large part of the experimental protocol related to the evaluation of the effect of KSL-W on C. albicans.
Funding Support:	

Name:	M. Abdelhabib Semlali
Project Role:	Post-Doc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	Five h a week
Contribution to Project:	M. Semlali has supervised the grad student.
Funding Support:	Laval University Foundation

Name:	Abdullah Alamri
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	University Laval student
Nearest person month worked:	5
Contribution to	M. Alamri contributed, with the grad student M. Teberge to

Project:	perform the genes expression protocols and data collection and analyses.
Funding Support:	

Name:	Leung KP
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	5
Contribution to Project:	K. Leung has contributed the study design and manuscript revision.
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report.

The Organizations involved as partners

The University Laval as an involved organization.

At the dental Faculty of Laval University, I was able to use different equipment to perform the study and get publishable results. Without such in-kind supports, the study would be very difficult/impossible to realize. The equipments at the research center of the Dental Faculty at Laval University were obtained because of the financial supports of University Laval and different funds that Dr Rouabhia obtained previously from different funding agencies. These include the CIHR, NSERC, FRSQ, the fonds Émile-Beaulieu at the dental Faculty of Laval University, and so.

8-SPECIAL REPORTING REQUIREMENTS

We need a no cost extension to perform what left from the study. The Pi and the University of Laval will send scientific and financial final reports to Ms. Wendy Baker and/or Mr. Robert Jones. We would like to have a 24 months no cost extension because the original agreement ended in 2014. This should give us time to tie all the loose ends of the study to have final reports on time.

9-APPENDICES:

Appendix 1: Published paper
Appendix 2: Presented abstracts (1, 2 and 3).